EXHIBIT 6a

UNITED STATES DISTRICT COURT
SOUTHERN DISTRICT OF NEW YORK

IN RE: Acetaminophen – ASD-ADHD Products Liability Litigation **This Document Relates To: All Actions**	**22:md-3043 (DLC)**

EXPERT REPORT OF ROBERT M. CABRERA, Ph.D.

TABLE OF CONTENTS

The purpose of this report is to examine the developmental and reproductive toxicity of acetaminophen, also known as paracetamol, and sold as a medication used to treat fever and pain under common brand names such as Tylenol and Panadol. The capacity of this substance and its metabolites to cause general toxicity, neurotoxicity, and development and reproductive toxicity will be addressed using standard methodology for causality analysis.

The opinions set forth herein are expressed with a reasonable degree of scientific certainty. Moreover, the opinions stated in this report are consistent with the totality of available evidence on acetaminophen and its metabolites. This evidence includes both public and confidentially disclosed data, reports, and presentations.

QUALIFICATIONS

Robert M. Cabrera, Ph.D.

I have studied and conducted birth defect research for over 20 years, as indicated in my curriculum vitae, attached hereto as Appendix A. I work in the field of science called teratology. Teratology is the scientific study of abnormalities, malformations, and developmental disorders that occur during prenatal development. It is a branch of developmental biology and embryology that focuses on understanding the causes, mechanisms, and outcomes of birth defects. The field of teratology examines the impact of various factors on the development of embryos and fetuses, including genetic abnormalities, environmental agents, maternal health conditions, and drug exposures. By studying these factors and their effects, teratologists aim to identify the causes of birth defects and develop strategies for prevention and treatment.

Teratology encompasses overt toxicity, including environmental or chemical exposures that produce death of an embryo or fetus, and a wide range of abnormalities, such as structural defects (e.g., cleft lip or heart malformations), growth abnormalities (e.g., small for gestational age or intrauterine growth restriction), and functional impairments (e.g., neurodevelopmental disorders or sensory disorders). There are termed the four manifestations of deviant development (i.e., death, malformations, growth retardation, and functional deficits). Researchers in this field often use animal models, such as mice, rats, rabbits, or zebrafish, to investigate the underlying mechanisms of abnormal development and to test potential interventions. In the section below titled "Embryology and Teratology" I provide additional details on these subjects.

The goal of teratology is to enhance our understanding of normal and abnormal development, promote healthy pregnancies, and minimize the risk of birth defects through education, research, and public health initiatives. My primary research interests include the teratogenicity of environmental exposures and pharmaceuticals, and the study of human and animal teratogens has been central to my training, academic research, and teaching activities.

I am currently an Associate Professor in the Center for Precision Environmental Health, Department of Cellular and Molecular Biology, at Baylor College of Medicine in Houston. I maintain an adjunct appointment with San Jacinto College, Department of Biology, in Houston. I established the online Nutrition course at The University of Texas at Austin, where I also held an adjunct appointment with University Extension. My prior appointments in Austin included Instructor at Concordia University, Manager for Stem Cell Research at the Dell Pediatric Research Institute, and a Faculty Lecturer and Research Scientist in the Department of Nutritional Sciences at The University of Texas at Austin. I have

a Ph.D. in Medical Sciences from Texas A&M University Health Science Center, where I studied genetic, immunologic, and pharmaceutically induced congenital malformations in experimental animal models and humans.

I obtained my undergraduate degree at Texas A&M University in College Station (B.A., 1995). I have worked in private industry laboratories doing genetic sequencing of humans, animals, plants, bacteria, and viruses, and I have developed commercial processes for using high-content screening of genetic polymorphisms and gene expression analyses for clinical applications. I enrolled in graduate studies at Texas A&M University Health Science Center and completed my graduate training at the Institute of Biosciences and Technology in Houston, Texas (Ph.D., 2006). My graduate mentor was Professor Richard H. Finnell, who is a leader in the field of teratology and has worked in the area of birth defects research for over 35 years as a board-certified medical geneticist and as an established animal research investigator.

A major theme of my work is the complex and often reciprocal interrelationships between maternal immunity and birth defect risks. My graduate school research focused on developing and utilizing mouse models for birth defect research and developing novel laboratory tests for measuring risk factors for birth defects from the California Birth Defects Monitoring Program.[1] This included animal mechanistic studies using genetic manipulation, toxicant exposures, and studies on valproic acid (Depakote), a recognized human teratogen, and one of the most widely prescribed anti-epileptic medications. My post-doctoral work included the study of associations between mothers with folate receptor autoimmunity and the risk of neural tube defects (NTDs). These studies examined paradigms in developmental biology, including the reduced risk for neural tube defects through supplementation with folic acid, and disrupted fertility, placental function, and embryo-fetal development due to autoimmunity. In several studies, I found that maternal IgG or IgM autoantibodies to folate receptors increase the risk for NTDs. I also determined IgM autoantibodies were associated with risks for cleft palate and NTDs in a Norwegian population. Specifically, my postdoctoral research involved the continued study of birth defects in expectant mothers from the Norwegian Mother and Child Cohort Study[2] (MoBa) and the Danish National Birth Cohort Study.[3] The Norwegian Mother and Child Cohort Study (MoBa) is a large population-based study conducted in Norway. It is one of the largest pregnancy cohort studies in the world and has been ongoing since 1999. The primary aim of MoBa is to investigate various factors that influence health and development in pregnancy, infancy, childhood, and beyond. The study involves the participation of pregnant women in Norway who are recruited during their routine ultrasound examination at approximately 17-18 weeks of gestation. The women provide information through comprehensive questionnaires during pregnancy and after birth. The Danish National Birth Cohort (DNBC) Study, also known as "Danish Mother and Child Cohort Study," is a large-scale population-based study conducted in Denmark. The DNBC recruited pregnant women from 1996 to 2002, enrolling over 100,000 participants. The study involved collecting detailed information through comprehensive questionnaires completed by the pregnant women at different stages of pregnancy, during birth, and following birth. Additionally, biological samples such as blood and urine were collected from the participants. I analyzed autoimmune

---

[1] Cabrera et al. Autoantibodies to folate receptor during pregnancy and neural tube defect risk. J Reprod Immunol. 2008 Oct;79(1):85-92. doi: 10.1016/j.jri.2008.08.002. Epub 2008 Sep 18. PMID: 18804286; PMCID: PMC3998370.

[2] Boyles et al. Association between inhibited binding of folic acid to folate receptor alpha in maternal serum and folate-related birth defects in Norway. Hum Reprod. 2011 Aug;26(8):2232-8. doi: 10.1093/humrep/der144. Epub 2011 May 15. PMID: 21576080; PMCID: PMC3137385.

[3] Bille et al. Autoantibodies to folate receptor alpha during early pregnancy and risk of oral clefts in Denmark. Pediatr Res. 2010 Mar;67(3):274-9. doi: 10.1203/PDR.0b013e3181cbd564. PMID: 19952865; PMCID: PMC2909840.

factors in patients from these populations as a risk factor for various birth defects, including neural tube defects and cleft lip and palate. These study populations have also been used to investigate other risk factors for birth defects and functional deficits, including autism spectrum disorder (ASD) and attention-deficit hyperactivity disorder (ADHD).

Upon completion of my postdoctoral research, I worked for a non-profit organization, the Texas Institute for Genomic Medicine (TIGM). TIGM was a public-private partnership that created and distributed mouse embryonic stem cell clones with genes inactivated (knockouts) to investigators around the world. As a Research Scientist at TIGM, I created animal models of human diseases and developed embryonic stem cell models for testing developmental and biological select agents and toxins. My project proposal was awarded $12.25 million dollars through the Defense Threat Reduction Agency (DTRA, US DOD) to study select agents using the testing models I developed. TIGM was subsequently assimilated entirely into the Texas A&M University System. I transitioned to The University of Texas at Austin and the Dell Pediatric Research Institute as a Research Fellow, where I continued my research on mouse and embryonic stem cell models for reproductive and developmental toxicity testing. During my academic career, I have written chapters on the use of functional genetics in developmental toxicity testing and have published over 30 peer-reviewed articles on teratogens and birth defects. I am currently developing and maintaining National Institutes of Health and National Institutes of Mental Health funded research programs that lead the field in developmental toxicity testing of anti-retroviral therapies (ART) at Baylor College of Medicine. In this capacity, I have provided oral and written reports to national (FDA, CDC) and international (WHO) regulatory agencies on the prevention of birth defects associated with ART. I am also on the editorial advisory board for Birth Defects Research, the peer-reviewed journal of the Birth Defects Research and Prevention Society (Teratology Society). Through my experience in teratology research, I have examined and processed hundreds of litters, and thousands of fetuses, for external, visceral, and skeletal malformations. I actively conduct and participate in human epidemiology and animal teratology studies that focus on determining the mechanisms of birth defects. This includes the use of clinical chemistry, analytical chemistry, immunological testing and immunodiagnostics, pathology, and hematology. I also conduct and participate in environmental chemical and pharmaceutical studies for teratogenicity testing using cellular, tissue, organ, and *in vivo* models, including zebrafish, genetically altered mice, and inbred mice.

My teaching activities include lectures, laboratory training and instruction, directing projects, and mentoring or assisting student researchers. My current teaching load includes professional student and graduate student lecture/seminar courses in the field of medical biochemistry, in the School of Health Professionals, and guest lecturing in developmental biology and nutritional sciences. I am also a course instructor at Baylor College of Medicine and participate in the development/design of the medical biochemistry curriculum.

Based on my qualifications, training, and experience, I am competent to set forth the opinions below.

SUMMARY OF OPINIONS

After performing a Weight of Evidence (WoE) analysis: reviewing the chemical profile of acetaminophen (APAP), regulatory adverse outcome pathway (AOP), and the preclinical, *in vitro, ex vivo*, and *in vivo* studies for potential reproductive, developmental, and neurodevelopmental effects, in the context of "therapeutic" doses of APAP use;

I hold the following opinions to a reasonable degree of scientific certainty:

I. Acetaminophen produces oxidative damage, reproductive, developmental, and neurodevelopmental toxicity at clinically relevant dosages, including neurodevelopmental disorders, such as autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD).

II. Therapeutic dosages of acetaminophen taken by pregnant woman are sufficient to cause neurotoxicity, neurodevelopmental disorder, ASD, and ADHD in exposed offspring.

III. There is a historic and growing body of research and evidence in the scientific and medical community that support the reproductive, developmental, and neurodevelopmental toxicity of acetaminophen exposures.

Additional opinions and subsidiary opinions are also expressed throughout this report.

MATERIALS CONSIDERED

In order to convey my opinions, I will present the relevant data and available information:

1. My knowledge, training, and experience as set forth above and in my annexed curriculum vitae;

2. Disclosures provided by Defendant(s) including responses to discovery demands identifying research, presentations, and reports on acetaminophen;

3. The scientific and medical literature cited throughout this report;

4. Governmental and regulatory publications cited throughout this report;

5. Other expert reports submitted by Plaintiffs, including expert reports prepared by Drs. Andrea Baccarelli, and Brandon Pearson; and

6. Depositions and documentary disclosure provided in these cases.

SUMMARY OF EVIDENCE

The following summary is based on a systematic review of relevant studies on acetaminophen toxicity and guidance documents on reproductive, developmental, and neurodevelopmental toxicity testing. **The primary question to be answered: does acetaminophen exposure during pregnancy cause functional deficits in offspring, specifically, ASD and ADHD?** The population studied is pregnant females. The exposure of interest is pharmaceutical intake of acetaminophen during gestation. These exposures are compared to females unexposed or as a dosed-response exposure, and the outcomes examined include manifestations of deviant development, generally, and functional deficits, specifically, ASD and ADHD. The protocol followed is based on Weight of the Evidence (WoE) for examining study quality and Bradford Hill, applied for significant associations, to evaluate causality. A comprehensive literature search was conducted, and relevant information and studies are summarized below and detailed in the following report. Background information is provided and reviews the pharmacokinetic, mechanism of action, and associated toxicities with acetaminophen exposures. Both primary reports from animal model testing and epidemiology studies were reviewed. In addition, meta-analyses were reviewed, and alternative explanations were considered. I have interpreted the collective totality of evidence, identified associations, strengths, and limitations in studies, and draw the following conclusion: **based on the totality of evidence, acetaminophen exposure during pregnancy causes ASD and ADHD in offspring.**

Summary of Toxicological Endpoints and Risk Characterization for Acetaminophen

Topic	Key Information, Data, and Evidence
Pharmacokinetics of Acetaminophen (APAP)	•Available in tablet, capsule, intravenous infusion, and liquid formulations (PDR) ·Bioavailability ranges by formulation from 85-98% ·Immediate release peak plasma concentrations occur within 30-60 minutes with oral administration and range from 7.7 to 17.6 mcg/mL (~50-116 µM) after a single 1,000 mg dose and 7.9 to 27 mcg/mL (~52-178 µM) at steady state after 1,000 mg every 6 hours in adult patients. ·In children 2 to 7 years of age, acetaminophen (12 mg/kg) achieved maximum concentration (14.6 +/- 2.6 mcg/mL) (~96 µM) within 0.55 +/- 0.08 hours.
Therapeutic Mechanism of Action	• **APAP is the only aniline analgesic still in use, all other members of this drug class are banned.** · Human therapeutic dose = 1g (16.7mg/Kg in 60Kg human), mouse median effective dose as antinociceptive (ED_{50}) = 125.4mg/Kg, rat ED_{50} = 154.8mg/Kg (Guash 1990). ·Several therapeutic mechanisms have been reported, including cyclo-oxygenase (COX) inhibition, serotonergic (5-HT) signaling, and metabolite mediated (AM404) cannabinoid signaling. ·AM404 activates TRPV1, a ligand at cannabinoid receptors (CB1) and an inhibitor of endogenous cannabinoid (anandamide) uptake.
General Toxicity	• **One of the most common causes of poisoning worldwide. (PubChem)** • **Most common cause of acute liver failure in the United States. (PDR)** ·Risk of toxicity increases with serum ≥150 µg/mL at 4 hours post ingestions, termed the "150" treatment line (~990 µM). • **Increased risk of acute oral overdose at intake ≥ 150 mg/kg (~7.5 g in adults)** ·Limited margin of safety comparing daily therapeutic 4g (1g/6 hours) and risk of acute toxicity at ≥ 7.5g. • **The toxic effects of APAP are due to a metabolite (NAPQI). (PubChem)** ·Cytochromes (P450) CYP2E1 and CYP3A4 convert ~5% of APAP to NAPQI. ·NAPQI reacts with sulfhydryl groups on proteins and with glutathione (GSH). ·GSH is an essential antioxidant; decreased GSH can result in protein, cell, tissue, and organ damage. • **Lethal Dose (LD) references for reviewed animals** ·Mouse LD50 = PO 945-1212 mg/kg (Guash 1990), IP 800mg/Kg (Mancini 1980) ·Rat LD50 = PO >4000 mg/kg (Guash 1990), IP 1580mg/Kg (Mancini 1980)

Topic	Key Information, Data, and Evidence
Genotoxicity and Carcinogenicity	• **Animal models and human studies report APAP increases leukemia risk (NTP, Weiss et al. 2005)** • **APAP is associated with an estimated two-fold increased risk (point estimate range: 1.5-2.3) of myeloid leukemias in humans. (Weiss et al. 2005, Walter et al. 2011)** • **There was equivocal evidence of carcinogenic activity of acetaminophen in female rats (F344/N) based on increased incidences of mononuclear cell leukemia. (NTP)** ·Equivocal evidence of carcinogenic activity describes studies that are interpreted as showing a marginal increase of neoplasms that may be chemically related. (NTP)
Adverse Outcome Pathway (AOP) for Neurodevelopmental Impairment	• **APAP readily crosses the placenta and the fetal blood-brain barrier.** ·Therapeutic doses of APAP produce the toxic metabolite NAPQI in sufficient concentrations to produce oxidative stress adverse effects in every region of the brain. • **Oxidative stress from NAPQI reduces/depletes the antioxidant glutathione (GSH)** ·Oxidative damage to developing neural tissues (dose and time dependent), results in dysregulated proliferation, differentiation, and patterning of neurons and neural cells. • **Therapeutic doses of APAP produce the metabolite AM404** ·Increased anandamide/endocannabinoid signaling. ·Disruption of endocannabinoid signaling during brain development results in neurodevelopmental impacts, including cognitive impairment of learning and memory, behavioral problems, and attention difficulties. • **Animal studies demonstrate that perinatal exposure to APAP at therapeutic doses cause significant changes in brain biochemistry, learning, and behavioral testing.** ·**Humans with ASD and ADHD have biomarkers, brain tissue patterns, and reproductive impacts consistent with outcomes observed in *in utero* exposure to APAP in animal models.**
Reproductive, Developmental, and Neurodevelopmental Toxicity	• **Epidemiological studies and meta-analysis report that use of APAP during pregnancy significantly increases risk of ASD and ADHD in offspring.** ·Studies measuring APAP in cord blood or meconium replicate and support dose-effects on neurodevelopment and reject recall bias. ·Confounding by indication or unknown confounding are proposed as alternative explanations for reported associations/risks. ·Studies comparing APAP and other analgesics to ASD or ADHD do not support confounding by indication. • **Exposing mice or rats to human equivalent doses of APAP during neural development causes altered behavior consistent with ASD and ADHD.** ·Reproductive, developmental, and neurodevelopment impacts on animals occur in the absence of indication. • **15 studies in animal models investigated the effects of early-life exposure to acetaminophen on behavior, with 14 showing impaired learning and altered behaviors.** • The one study that found "no evidence" showed low doses of acetaminophen improved learning. This study was conducted at sub-therapeutic dosing.

Weight of Evidence, Bradford Hill, and Causal Interactions	• The Weight of Evidence (WoE) analysis on available human and animal studies demonstrates "clear evidence" of neurodevelopmental toxicity at "therapeutic" doses. APAP and the metabolite NAPQI increase (1) oxidative damage, (2) DNA oxidation, (3) protein adduct formation, and thereby cause (4) epigenetic and associated gene expression changes, (5) mitochondrial and cellular toxicity, and (6) reproductive, developmental, and neurodevelopmental toxicity. • Based on Bradford Hill, there is sufficient data to support causal interactions between APAP and reproductive toxicity and neurodevelopmental toxicity, including ASD and ADHD. • Strength of Association: The meta-analyses indicate statistically significant associations between acetaminophen exposure and ASD and ADHD risk. These reports meet the criterion of strength of association. • Consistency: The meta-analyses combine data from multiple cohort studies, indicating a consistent pattern of association between acetaminophen exposure and ASD and ADHD risk across different populations. The consistency criterion is met. • Specificity: APAP exposure is common and is associated with other toxicities, such as being the number one cause of acute liver failure in the USA. ASD and ADHD can be caused by other environmental or chemical exposures, such as *in utero* valproic acid exposure. The specificity criterion is not fully met. • Temporality: The exposure to acetaminophen (during pregnancy) precedes the outcome (development of ASD or ADHD) temporally, supporting a temporal relationship. The temporality criterion is met. • Biological Gradient: The meta-regression analyses indicate that the association between APAP exposure and ASD risk increased with the child's age at follow-up and the mean duration of exposure. This supports a biological gradient, as a longer duration of exposure and older age were associated with a higher risk of ADHD. The biological gradient criterion is met for ASD. Meta-analyses did not explicitly report a dose-response relationship between APAP exposure levels and the risk of ADHD. Two studies looked at APAP in meconium, one supporting a dose-response interaction between APAP and ADHD. Another study looked at cord blood and supported a dose-response interaction. The biological gradient criterion for ADHD is supported. • Plausibility: There is biological plausibility for the association, as APAP can cross the placental barrier and affect fetal neurodevelopment. The published adverse outcome pathway (OECD 20) also supports a causal interaction between APAP and oxidative stress and neurodevelopmental adverse outcomes. The neurodevelopmental effects of acetaminophen on ADHD risk are biologically plausible and supported.

Topic	Key Information, Data, and Evidence
	• Coherence: The association between APAP exposure and ASD and ADHD is coherent with the existing knowledge of oxidative stress and potential for hepatotoxicity and neurotoxicity with APAP exposures. The coherence criterion is met. • Experiment: Conducting experimental studies on women of childbearing age to test chemical causality of birth defects or functional deficits is not ethically feasible, as it would involve exposing pregnant women to potentially harmful substances. Animal models report dose-responsive reproductive toxicity, dose-responsive developmental toxicity, and neurodevelopmental toxicities with perinatal APAP exposures. This criterion is met with animal models. • Analogy: There are analogies with other substances that are known to have neurodevelopmentally toxic effects during pregnancy, including mercury and valproic acid, supporting a common oxidative stress mechanism for mercury, like APAP, and chemical-pharmaceutical causes of ADHD and ASD.

Key Terms: APAP: paracetamol/acetaminophen (N-acetyl-p-aminophenol); NAPQI: APAP metabolite N-acetyl-p-benzoquinone imine; AM404: APAP metabolite N-arachidonoylphenolamine; COX: cyclooxygenase; 5-HT: serotonin; DART: developmental & reproductive toxicity; LD50: median lethal dose (dose that results in death of 50% of animals); OECD: The Organization for Economic Co-operation and Development; WoE: weight of evidence; NTP: National Toxicology Program; PDR: The Physicians' Desk Reference (acetaminophen - Drug Summary), PDR.net (online); ASD: Autism Spectrum Disorder; ADHD: Attention Deficit Hyperactivity Disorder.

- Guasch et al. Pharmaco-toxicological effects of acetaminophen in rodents. Battery of tests to screen potential analgesic acetaminophen derivatives. Methods Find Exp Clin Pharmacol. 1990 Mar;12(2):141-8. PMID: 2319838.

- Mancini, R et al. Developmental susceptibility to acetaminophen toxicity. Res Commun Chem Pathol Pharmacol. 1980 Mar;27(3):603-6. PMID: 7384649.

- Weiss et al. Opposing effects of aspirin and acetaminophen use on risk of adult acute leukemia. Leuk Res. 2006 Feb;30(2):164-9. doi: 10.1016/j.leukres.2005.06.023. Epub 2005 Aug 11. PMID: 16099041.

- Walter et al. Long-term use of acetaminophen, aspirin, and other nonsteroidal anti-inflammatory drugs and risk of hematologic malignancies: results from the prospective Vitamins and Lifestyle (VITAL) study. J Clin Oncol. 2011 Jun 10;29(17):2424-31. doi: 10.1200/JCO.2011.34.6346. Epub 2011 May 9. PMID: 21555699; PMCID: PMC3107756.

- National Toxicology Program. NTP Toxicology and Carcinogenesis Studies of Acetaminophen (CAS No. 103-90-2) in F344 Rats and B6C3F1 Mice (Feed Studies). Natl Toxicol Program Tech Rep Ser. 1993 Jan;394:1-274. PMID: 12637965.

METHODOLOGIES EMPLOYED

In performing my analyses, I have brought to bear the accepted methodologies that are commonly employed in medical and scientific disciplines, to assess general causation. These include the general principles of a) genetics and clinical genetics, b) toxicology, c) pharmacology d) epidemiology, e) epigenetics, and f) teratology and developmental toxicology.

Causality is a concept that has been studied for centuries. One of the most influential theories on causality was developed by Sir Bradford Hill in 1965. Hill's criteria for establishing causality have become known as the Bradford Hill criteria in scientific, medical, and legal writings. These criteria provide a framework for determining whether or not there is a causal relationship between two variables. The Bradford-Hill Criteria are as follows:

1. **Strength of association**: A small association does not mean that there is not a causal effect, though the larger the association, the more likely that it is causal.
2. **Consistency:** Consistent findings observed by different persons in different places with different samples and techniques strengthen the likelihood of an effect.
3. **Specificity**: Causation is likely if there is a very specific population at a specific site and disease with no other likely explanation. The more specific an association between a factor and an effect is, the bigger the probability of a causal relationship.
4. **Temporality**: The effect has to occur after the cause (and if there is an expected delay between the cause and expected effect, then the effect must occur after that delay).
5. **Biological gradient**: Greater exposure should generally lead to greater incidence of the effect. However, in some cases, the mere presence of the factor can trigger the effect. In other cases, an inverse proportion is observed: greater exposure leads to lower incidence.
6. **Biological plausibility**: A plausible mechanism between cause and effect is helpful (but Hill noted that knowledge of the mechanism is limited by current knowledge).
7. **Coherence**: Consistency with the known natural history and biology of the disease supports causation.
8. **Experiment**: "Occasionally it is possible to appeal to experimental evidence".
9. **Analogy**: The presence of an analogous drug-disease causal relationship supports a causal inference.

I have employed weight-of-the-evidence[4] and adverse outcome pathway analyses[5] in this report. This approach is consistent with various guidance documents provided by the those of the United States Environmental Protection Agency (USEPA) and Organisation for Economic Co-operation and Development (OECD) Developmental Neurotoxicity (DNT) Guidelines (OCSPP 870.6300 or OECD TG 426)[6] as well as OECD TG 443 (OECD, 2018)[7] which require testing of learning and memory, and OECD

[4] Weight-of-the-evidence means the review of clinical observations, case reports, epidemiological and animal studies, toxicological experiments, exposure data, industrial hygiene and engineering studies, genetic testing, radiological findings, biochemical experiments, embryological information, medical diagnoses, and similar information that bear upon questions of causation. The manner in which scientists go about evaluating this data, including the assessment of the internal and external validity of this data is called a weight-of-the-evidence approach.

[5] Adverse outcome pathway analyses consider data describing the adverse consequences of exposure to a toxin at the molecular, cellular, tissue, organ, whole body, and population levels in assessing questions of association and causality.

[6] Health Effects Test Guidelines, OPPTS 870.6300, Developmental Neurotoxicity Study

[7] No. 151 Guidance Document Supporting OECD Test Guideline 443 on the Extended One-Generation Reproductive Toxicity Test

GD 43 (2008)[8] on reproductive toxicity, and ICH S5(R3) Detection of Reproductive and Developmental Toxicity for Human Pharmaceutical (ICH 2020), S5(R3) Detection of Reproductive and Developmental Toxicity for Human Pharmaceuticals (FDA 2021), and Reproductive and Developmental Toxicities-Integrating Study Results to Assess Concerns (FDA 2011). I have also been informed by Teratology (Wilson's Principles),[9] Weight of the Evidence, and systematic review methodology regarding proof of teratogenicity, developmental and reproductive toxicology, neurotoxicity, and establishment of causality.

Weight of Evidence:

The integration of animal and human data to assess the potential risk of medication use in pregnant women is necessary for several reasons. The foremost barrier for testing directly in pregnant women, is the risk of toxicity to the unborn child that could result in injury, malformations, or death. This risk is mitigated by performing reproductive and developmental toxicity testing on rodent and non-rodent animals, often referred to as preclinical testing. The second line of evidence is from human epidemiological studies, but these data are only made available after a medication has been approved for use and is then used by pregnant women. Study quality assessments of both human and animal data are incorporated for the weight of evidence (WoE) analysis.[10]

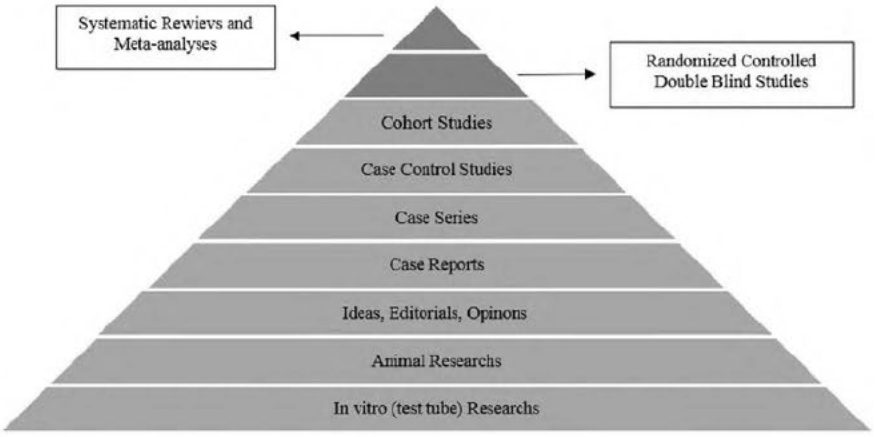

Figure 1. Hierarchy of Evidence.[11] The hierarchy of evidence categorizes studies to provide levels of evidence. It is used to classify the relevance of evidence to human health benefits or risks. The relevance is proposed to increase and potential for bias decrease at higher levels.

1. Animal Studies

Animal-based studies of developmental toxicology provide initial, generally accepted guidelines as to whether a compound, including a pharmaceutical drugor chemical, may present a teratogenic risk during pregnancy. The current testing guidelines provided by the FDA for reproductive toxicity rely upon the use of animal models to evaluate teratogenicity. Such testing procedures have the dual role of potentially identifying compounds that can interfere with normal development and elucidating the mechanism by

[8] No. 43 Guidance Document on Mammalian Reproductive Toxicity Testing and Assessment.

[9] The founding principles of the modern study of teratogens were initially articulated by James G. Wilson (1973), co-founder of The Teratology Society, in his monograph Environment and Birth Defects.

[10] Wurst et al. A model for human and animal data integration: Weight of evidence strategy. Birth Defects Res. 2020 Nov;112(18):1505-1512. doi: 10.1002/bdr2.1775. Epub 2020 Aug 7. PMID: 32770662.

[11] Reviewed in Murad MH, Asi N, Alsawas M, Alahdab F. New evidence pyramid. Evid Based Med. 2016 Aug;21(4):125-7. doi: 10.1136/ebmed-2016-110401. Epub 2016 Jun 23. PMID: 27339128; PMCID: PMC4975798; Figure from Evidence Based Practices in Health Sciences. ResearchGate. ISBN 978-954-07-4344-8.

which the dysmorphogenic events occur.

Using animal models is critically important in establishing the biological plausibility that a suspected compound could be a human teratogen. The physiological systems of mammals depend on common and conserved genetic, cellular, and tissue processes that havebeen shared and refined over time. Thus, the fundamental biology of how cells behave is shared among closely related species, allowing us to predict from animal studies what is expected to occur in humans.[12]

The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) has set forth generally accepted guidelines for testing the teratogenicity of a compound.[13] This testing is generally performed in two species, rabbits and rats. Additionally, mice are an acceptablealternative testing model that is used in place of rats. Tests using mice produce quality study models equally as predictive as rat designs. Moreover, as the mouse genome is comparable to humans, both in size and complexity, mice are very well suited for studies that mimic teratogenicexposures in humans.

Animal testing proceeds by exposing a pregnant animal (dam) to a test compound by an appropriate route of administration during a selected period of gestation. Some studies are restricted to the period of organogenesis, when the major organ systems of theembryo are forming. In humans, with the caveat that the date of "conception" and last menstrual period may be unclear in an individual female, weeks 2-8 post-conception were considered the period of organogenesis, although more recent terminology refers to the first eight weeks post-conception as the Embryonic Period.[14] In rodents, typically days 6-12 of gestation are tested, which encompass the major events of organogenesis. Other experimental paradigms might have the treatment start at fertilization or implantation, the time when the fertilized egg, now a blastocyst, attaches to the uterus, and the treatment is administered daily until the end of the selected study period.[15]

The physical examination of the soft tissues of embryos typically involves preparing histological sections of fixed fetuses,[16] although combinations of methods using fresh and fixed material are not uncommon.[15] For these studies, thefetal tissue is placed in fixative solution to cross-link proteins and thereby stiffen it so that it can be sliced by a razor blade and the thick pieces (sections) are examined under a microscope. To resolve pathology at a more sensitive level, the tissue is fixed, embedded in paraffin wax, and then thin (micrometer) sections are cut on a microtome. The terminology usedto describe the specific defects observed in the sectioned fetal tissue has been standardized to be descriptive, rather than diagnostic or interpretative.[17] The data generated from each study animal can be tabulated for statistical analyses,

---

[12] Daston. Laboratory models and their role in assessing teratogenesis. Am J Med Genet C Semin Med Genet. 2011 Aug 15;157C(3):183-7. doi: 10.1002/ajmg.c.30312. Epub 2011 Jul 15. PMID: 21766439.

[13] Cavagnaro. Preclinical safety evaluation of biotechnology-derived pharmaceuticals. Nat Rev Drug Discov. 2002 Jun;1(6):469-75. doi: 10.1038/nrd822. PMID: 12119749.

[14] Sadler and Langman (2012). Langman's medical embryology. Philadelphia, Wolters Kluwer Health/Lippincott Williams & Wilkins.

[15] Wise et al. Embryo-fetal developmental toxicity study design for pharmaceuticals. Birth Defects Res B Dev Reprod Toxicol. 2009 Dec;86(6):418-28. doi: 10.1002/bdrb.20214. PMID: 20025038.

[16] Wilson. (1973). Environment and birth defects. New York,, Academic Press.

[17] Makris et al. Terminology of developmental abnormalities in common laboratory mammals (version 2). Reprod Toxicol. 2009 Nov;28(3):371-434. doi: 10.1016/j.reprotox.2009.06.010. Epub 2009 Sep 1. Erratum in: Reprod Toxicol. 2012 Nov;34(3):487. PMID: 19729062.

including data on: pre-implantation loss (the death of embryos from the time they are conceived until just before they implant in the uterus), resorptions (fetuses that implanted, developed for some time, then died and all that remains is a small clot of disorganized tissue), dead fetuses, live fetuses, fetal weight and the fetal evaluations. The analyses should make comparisons to the concurrent control group, although it can be useful to refer back to historical control data at times.[15] In assessing the weight-of-the-evidence for each animal study, I employed the six Quality Assessment Point for animal studies:

The Six Quality Assessment Points to be Evaluated in Animal Studies:[18]

Animal data	
Point to evaluate	**Areas of evaluation**
Test article	• Source • Purity • Vehicle • Evidence of accurate formulation • Evidence of stability appropriate for study
Test system	• Species • Gender • Age range • Husbandry information
Control group	• Vehicle and/or positive control
Study design or purpose	• Number of animals • Route of administration • Exposure information including dosage and duration • Statistical methods
Endpoints	• If appropriate for evaluation of study objective • If not standard, rationale provided
Results	• Presented transparently • Clear/correct identification of test article-related effects • Conclusions are reasonable based on the study design and results

[18] Wurst et al. A model for human and animal data integration: Weight of evidence strategy. Birth Defects Res. 2020 Nov;112(18):1505-1512. doi: 10.1002/bdr2.1775. Epub 2020 Aug 7. PMID: 32770662.